NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF OXAZEPAM

(CAS NO. 604-75-1)

IN F344/N RATS

(FEED STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

October 1998

NTP TR 468

NIH Publication No. 99-3958

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

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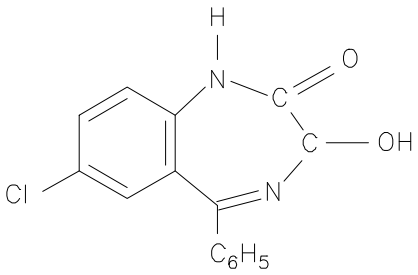
CONTENTS

ABSTRACT



OXAZEPAM

CAS No. 604-75-1

Chemical Formula: $C_{15}H_{11}ClN_2O_2$ Molecular Weight: 286.74

Synonym: 7-Chloro-1,3-dihydro-3-hydroxy-5-phenyl-2*H*-1,4-benzodiazepin-2-one
Trade Names: Serax, Tazepam, Wy-3498

Oxazepam and related benzodiazepine drugs are used in the treatment of anxiety. All benzodiazepines currently in use share a number of effects, including sedation, hypnosis, decreased anxiety, muscle relaxation, amnesia, and anticonvulsant activity. Oxazepam and four other benzodiazepines (chlordiazepoxide, chlorazepate, diazepam, and flurazepam) were nominated for study by the Food and Drug Administration (FDA) and by the NIEHS based on their widespread use, use by pregnant women, and the lack of adequate rodent carcinogenicity studies. Oxazepam was evaluated in 14-week and 2-year studies by the NTP, and Technical Report No. 443 contains the results of the studies performed with the Swiss-Webster and B6C3F$_1$ strains of mice. Studies with rats were not initiated at the same time as the mouse studies because adequate carcinogenicity studies of oxazepam with the Sprague-Dawley rat strain had been submitted to the FDA. Subsequently, because of the marked neoplastic responses found in the two mouse strains, the NTP initiated 2-year studies of oxazepam with the F344/N rat. Groups of male and female F344/N rats were exposed to oxazepam (greater than 99% pure) in feed for 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and cultured Chinese hamster ovary cells, and mouse peripheral blood samples were analyzed for the frequency of micronucleated normochromatic erythrocytes.

2-YEAR STUDY

Groups of 50 male and 50 female F344/N rats were fed diets containing 0, 625, 2,500, or 5,000 ppm oxazepam for up to 105 weeks. A stop-exposure group of 50 males and 50 females received 10,000 ppm oxazepam in feed for 26 weeks, after which animals received undosed feed for the remainder of the 2-year study. The continuous-exposure concentrations resulted in average daily doses of 25, 100, or 250 mg oxazepam/kg body weight to males and 25, 110, or 220 mg/kg to females. Stop- exposure males and females received an average daily dose of 630 mg/kg during the exposure period.

Survival, Body Weights, and Clinical Findings

All 5,000 ppm continuous-exposure and 10,000 ppm stop-exposure males died before the end of the study. Survival of 2,500 ppm continuous-exposure males and females was significantly less than that of the controls. The mean body weight gains of 2,500 and 5,000 ppm males and females were less than those of the controls throughout the study. The mean body weights of 10,000 ppm stop-exposure males were generally less than those of the controls throughout the study; those of 10,000 ppm stop-exposure females were less than those of the controls during the exposure portion of the study but increased steadily after the cessation of dosing at week 27. Feed consumption by exposed groups was similar to that by the controls after week 1 of the study. Treatment-related eye/nasal discharge, hyperactivity when handled, and/or ataxia were observed in exposed male and female rats on or about day 2 of exposure but were no longer apparent after day 7.

Plasma Oxazepam Determinations

Plasma oxazepam concentrations were measured at the end of the study. The concentrations ranged from approximately 0.5 (625 ppm males) to 2.8 μg/mL (5,000 ppm females).

Pathology Findings

In the standard histopathologic evaluation, the incidence of renal tubule adenoma was slightly increased in male rats exposed to 2,500 ppm and was at the upper limit of the historical control range for this neoplasm in 2-year NTP feed studies. In an extended evaluation (step section) of the kidneys of male rats, the incidences of renal tubule adenoma occurred with a positive trend in exposed groups. In standard and step sections (combined), male rats exposed to 2,500 or 5,000 ppm showed a significant increase in the incidences of renal tubule adenoma and hyperplasia. In addition, the incidences of renal tubule adenoma and hyperplasia were significantly increased in the 10,000 ppm stop-exposure group. The incidences of nephropathy in continuously exposed female rats were significantly greater than in the controls, and the severity of nephropathy increased with increasing exposure concentration in males.

The incidences of epithelial hyperplasia and chronic inflammation of the forestomach in males exposed to 2,500 and 5,000 ppm and of ulcers in 2,500 ppm males were significantly greater than in the controls. Incidences of mineralization of the glandular stomach in 5,000 ppm and 10,000 ppm (stop-exposure) males and of erosion of the duodenum in 5,000 ppm males were significantly greater than in the controls. Female rats exposed to 2,500 ppm had greater incidences of epithelial hyperplasia, chronic inflammation, and ulcers of the forestomach and of erosion in the glandular stomach.

Centrilobular hepatocyte hypertrophy occurred more frequently in 2,500 and 5,000 ppm males and females than in the controls.

GENETIC TOXICOLOGY

Oxazepam was not mutagenic in any of several strains of *S. typhimurium*, nor did it induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells. These *in vitro* tests were performed with and without S9 metabolic activation. Results from an *in vivo* mouse peripheral blood micronucleus test performed on B6C3F_1 mice used in a 14-week study were also negative.

CONCLUSIONS

In summary, under the conditions of these 2-year dosed-feed studies, there was *equivocal evidence of carcinogenic activity** in male F344/N rats, based on small increases in the incidences of renal tubule adenomas in exposed groups also exhibiting significantly enhanced nephropathy. There was *no evidence of carcinogenic activity* of oxazepam in female F344/N rats exposed to feed containing 625, 2,500, or 5,000 ppm for 2 years or 10,000 ppm for 6 months.

Administration of oxazepam to rats resulted in nonneoplastic lesions in the forestomach, glandular stomach, and small intestine as well as centrilobular hypertrophy of hepatocytes in the liver. In addition, nephropathy was increased in incidence in female rats and was markedly increased in severity in male rats, resulting in early mortality at the higher exposure concentrations.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Oxazepam

	Male F344/N Rats		Female F344/N Rats	
	(2-Year Study)	**(Stop-Exposure Study)**	**(2-Year Study)**	**(Stop-Exposure Study)**
Concentrations	0, 625, 2,500, or 5,000 ppm	10,000 ppm for 26 weeks	0, 625, 2,500, or 5,000 ppm	10,000 ppm for 26 weeks
Body weights	2,500 ppm and 5,000 ppm groups less than control group	10,000 ppm group generally less than control group	2,500 and 5,000 ppm groups less than control group	10,000 ppm group similar to control group
2-Year survival rates	17/50, 11/50, 6/50, 0/50	0/50	32/50, 26/50, 20/50, 31/50	25/50
Nonneoplastic effects	Kidney: severity of nephropathy (1.9, 2.3, 2.7, 3.2) Forestomach: chronic inflammation (6/50, 8/48, 23/50, 15/50); ulcer (9/50, 12/48, 20/50, 10/50); epithelial hyperplasia (5/50, 8/48, 25/50, 16/50) Glandular stomach: mineralization (0/50, 3/48, 1/50, 4/50) Small intestine: duodenum, erosion (4/50, 3/48, 9/49, 16/50) Liver: hepatocyte centrilobular hypertrophy (0/50, 1/50, 8/49, 14/50)	Kidney: severity of nephropathy (3.3) Forestomach: chronic inflammation (10/49); ulcer (7/49); epithelial hyperplasia (15/49) Glandular stomach: mineralization (16/47)	Kidney: nephropathy (32/50, 43/50, 41/50, 48/50) Forestomach: chronic inflammation (1/50, 5/50, 16/50, 3/50); ulcer (1/50, 2/50, 9/50, 6/50); epithelial hyperplasia (2/50, 6/50, 16/50, 5/50) Glandular stomach: erosion (0/50, 4/50, 7/50, 2/50) Liver: hepatocyte centrilobular hypertrophy (0/50, 0/50, 10/50, 31/50)	Forestomach: chronic inflammation (5/50); ulcer (4/50); epithelial hyperplasia (5/50)
Neoplastic effects	None	None	None	None
Uncertain findings	Kidney: renal tubule adenoma (extended evaluation - 1/50, 1/50, 4/50, 5/50; standard and extended evaluations combined - 2/50, 1/50, 7/50, 6/50)	Kidney: renal tubule adenoma (extended evaluation - 6/45; standard and extended evaluations combined - 6/45)	None	None
Level of evidence of carcinogenic activity	Equivocal evidence		No evidence	
Genetic toxicology				
Salmonella typhimurium gene mutations:	Negative with and without S9 in strains TA97, TA98, TA100, TA102, and TA1535			
Sister chromatid exchanges Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9			
Chromosomal aberrations Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9			
Micronucleated normochromatic erythrocytes in $B6C3F_1$ mice:	Negative at 14 weeks			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on oxazepam on 11 December 1996 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 11 December 1996, the draft Technical Report on the toxicology and carcinogenesis studies of oxazepam received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.R. Bucher, NIEHS, introduced the toxicology and carcinogenesis studies of oxazepam by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplastic and nonneoplastic lesions in rats. Dr. Bucher noted that the Subcommittee had reviewed a bioassay of oxazepam in Swiss-Webster and $B6C3F_1$ mice in 1993. The proposed conclusions for the present study were *some evidence of carcinogenic activity* in male F344/N rats and *no evidence of carcinogenic activity* in female F344/N rats.

Dr. Taylor, a principal reviewer, agreed in principle with the proposed conclusions. He stated that because there was substantial reduction in body weight gain and survival in 2,500 and 5,000 ppm male rats along with a dose response that was not very dramatic, he would argue for changing the proposed conclusion in male rats to *equivocal evidence of carcinogenic activity*. Noting the figure illustrating the metabolism of oxazepam in F344 rats, Dr. Taylor stated that some discussion of metabolism in rats and mice would be useful as an aid in trying to explain the difference between the mouse and rat in sites of toxicity and neoplasia.

Dr. Brown, the second principal reviewer, agreed with the proposed conclusions. He said that it would be helpful if additional information could be provided in the Abstract regarding the background against which this bioassay was conducted, i.e., the unpublished study by industry in Sprague-Dawley rats. Dr. Bucher observed that the 1996 edition of the *Physicians' Desk Reference* provides a description of the rat study performed by Wyeth Laboratories, although no doses are listed. The citation indicates that there were increased incidences of prostate adenoma, interstitial cell adenoma of the testes, and thyroid gland follicular cell adenoma, none of which were replicated in the current study in F344/N rats. Dr. W.R. Allaben, NCTR/FDA, pointed out that the data are considered proprietary information and by law cannot be released publicly. Dr. Bucher mentioned that this was one of four benzodiazepines nominated and selected for study. Three were products of Hoffmann-LaRoche, which agreed to carry out the studies with the NTP's assistance in the study design. The studies were completed, and data were submitted to the FDA.

There was a discussion about the appropriateness of step sectioning kidneys in male rats and about the exposure concentrations used in the study. Dr. Bucher said that in retrospect, the 1,250 ppm group, which was terminated after 26 weeks with the thought that it would be uninformative, would have been the best high exposure group. Dr. LeBoeuf argued that if the 5,000 ppm group were excluded from analysis because of very poor survival, and if the results for renal tubule adenomas in the 625 and 2,500 ppm groups were compared with those in the controls, then he would conclude that there was *equivocal evidence of carcinogenic activity*. Dr. J.K. Haseman, NIEHS, noted that the increased incidence of renal tubule adenoma in the 2,500 ppm group was significant at $P = 0.018$. Dr. Goldsworthy asked under what circumstances the NTP would consider a study to be inadequate for evaluation. Dr. Bucher said that, generally, the NTP might consider a study to be inadequate if there is poor survival and if there is no neoplasm response, such that the ability of the study to detect a response may be compromised. Dr. Goldsworthy asked whether excluding a 13-week study would occur more often in future bioassays. Dr. G.A. Boorman, NIEHS, pointed out that in the case of oxazepam, a 26-week study did not predict very well; however, the decision of whether to employ a 13-week study would have to be determined on a case-by-case basis by drawing on other available toxicity information.

Dr. Taylor moved that the Technical Report on oxazepam be accepted with the revisions discussed and the conclusion as written for female rats, *no evidence of carcinogenic activity*, but changed for male rats, from *some evidence of carcinogenic activity* to *equivocal evidence of carcinogenic activity*.

Dr. Brown seconded the motion. In discussion, Dr. Ward stated that having toxicity in an organ such as the kidney, where there were also neoplasms, strengthened the evidence for the neoplasms being chemically induced because the organ is a target site for the chemical. Dr. Taylor's motion was accepted with five yes votes to three no votes (Goldsworthy, Reddy, and Ward).